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Neither Folic Acid Supplementation nor Pregnancy Affects the Distribution of Folate Forms in the Red Blood Cells of Women[1–,3]

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Abstract

It is not known whether folate metabolism is altered during pregnancy to support increased DNA and RNA biosynthesis. By using a state-of-the-art LC tandem mass spectrometry technique, the aim of this study was to investigate differences in RBC folate forms between pregnant and nonpregnant women and between nonpregnant women consuming different concentrations of supplemental folic acid. Forms of folate in RBCs were used to explore potential shifts in folate metabolism during early erythropoiesis. Total RBC folate and folate forms [tetrahydrofolate; 5-methyltetrahydrofolate (5-methyl-THF); 4α-hydroxy-5-methyl-tetrahydrofolate (an oxidation product of 5-methyl-THF); 5-formyl-tetrahydrofolate; and 5,10-methenyl-tetrahydrofolate] were measured in 4 groups of women ($n = 26$): pregnant women (PW) (30–36 wk of gestation) consuming 1 mg/d of folic acid, and nonpregnant women consuming 0 mg/d (NPW-0), 1 mg/d (NPW-1), and 5 mg/d (NPW-5) folic acid. The mean ± SD RBC folate concentration of the NPW-0 group (890 ± 530 nmol/L) was lower than the NPW-1 (1660 ± 350 nmol/L) and NPW-5 (1980 ± 570 nmol/L) groups as assessed by microbiologic assay ($n = 26$, $P < 0.0022$). No difference was found between the NPW-1 and NPW-5 groups. We detected 5-methyl-THF [limit of detection (LOD) = 0.06 nmol/L] in all groups and tetrahydrofolate (LOD = 0.2 nmol/L) in most women regardless of methylenetetrahydrofolate reductase genotype. Most women consuming folic acid supplements had detectable concentrations of 5,10-methenyl-tetrahydrofolate (LOD = 0.31 nmol/L). However, there was no difference in the relative distribution of 5-methyl-THF (83–84%), sum of non-methyl folates (0.6–3%), or individual non-methyl folate forms in RBCs across groups. We conclude that although folic acid supplementation in nonpregnant women increases RBC total folate and the concentration of individual folate forms, it does not alter the relative distribution of folate forms. Similarly, distribution of RBC folate forms did not differ between pregnant and nonpregnant women. This trial was registered at clinicaltrials.gov as NCT01741077.

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[3]Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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Introduction

Requirements for folate are elevated during pregnancy because of the increased demand for purines and pyrimidines to facilitate rapid RNA and DNA biosynthesis; the transfer of one-carbon units via tetrahydrofolate, 10-formyl-tetrahydrofolate, and 5,10-methylenetetrahydrofolate is key in these anabolic pathways (1). In a competing pathway, 5-methyl-methyltetrahydrofolate (5-methyl-THF)[7] facilitates remethylation of homocysteine to produce methionine, which is then converted to *S*-adenosylmethionine, the universal methyl donor in the body (1). The metabolic changes that occur in pregnancy to accommodate elevated folate requirements are not fully understood.

Periconceptual folic acid supplementation and folic acid fortification of enriched cereal grain products have been mandatory in North America since 1998 and have resulted in a 26–47% reduction in neural tube defects (2–4). The Institute of Medicine (5) recommends that women able to become pregnant continue to consume 400 μg/d of folic acid from fortified foods and/or supplements, and at least 90% of North American women report taking prenatal vitamins containing folic acid during pregnancy (6–8). In Canada, available prenatal supplements contain either 1 or 5 mg of folic acid. Although recognition of the benefits of folic acid in the prevention of birth defects is acknowledged as a tremendous public policy success, some published accounts in the literature have associated folic acid supplementation during pregnancy with the risk of asthma, respiratory infection, wheezing, and central adiposity and insulin resistance in offspring; however, at least as many studies find no such association (9–11). An understanding of the physiologic shifts in folate metabolism that are associated with pregnancy and/or folic acid supplementation may improve our understanding of how maternal folic acid supplementation may influence the health of offspring both short- and long-term. Physiologic shifts in folate metabolism including in RBCs (reflecting folate metabolism earlier in their genesis) are well described in the literature secondary to dietary deficiencies, environmental exposures, and disease (12–18). We hypothesize that a shift toward nonmethylated forms of folate (e.g., tetrahydrofolate and formyl-tetrahydrofolate) during pregnancy may be one of the mechanisms to accommodate increased DNA and RNA biosynthesis.

There are several studies on the forms of folate in serum and RBCs of nonpregnant adults. It is estimated that 85–100% and 0–11% of the naturally occurring folate in serum is in the 5-methyl-THF and tetrahydrofolate forms, respectively (19–21). The distribution of RBC folate forms reported in both males and premenopausal women suggests that 87–100% of the RBC folates are in the 5-methyl-THF form with a small percentage as tetrahydrofolate (0–15%) and 5,10-methenyl-tetrahydrofolate (0–2%) (22–26). Currently, the distribution of folate forms in RBCs of pregnant women and in nonpregnant women consuming various doses of folic acid supplements has largely been unexplored.

[7]Abbreviations used: LC-MS/MS, LC tandem MS; LOD, limit of detection; MeFox, 4α-hydroxy-5-methyl-tetrahydrofolate; *MTHFR*, methylenetetrahydrofolate reductase; MVM, multivitamin and mineral supplement; NPW, nonpregnant women; NPW-0, nonpregnant women not consuming a folic acid supplement; NPW-1, nonpregnant women consuming a 1-mg/d folic acid supplement; NPW-5, nonpregnant women consuming a 5-mg/d folic acid supplement; PW, pregnant women; SPE, solid-phase extraction; 5-methyl-THF, 5-methyltetrahydrofolate.

The purpose of this study was to compare the distribution of folate forms in RBCs of pregnant and nonpregnant women and among nonpregnant women (NPW) consuming 3 different concentrations of supplemental folic acid (0, 1, or 5 mg/d) by using a new and improved state-of-the-art LC tandem MS (LC-MS/MS) technique (27–29). Understanding how pregnancy and folic acid supplementation influence the intracellular distribution of folate may help elucidate any metabolic adaptations that occur during reproduction or with pharmacologic doses of folic acid.

Methods

Participants

The study protocol was approved by the Research Ethics Board of the Hospital for Sick Children and informed written consent was secured. Women were excluded from participation if they had a preexisting medical condition or were consuming medications known to interfere with folate absorption or metabolism. Thirty-two healthy, nonsmoking women (aged 20–41 y) were recruited from staff and visitors at the Hospital for Sick Children in Toronto, Canada, between January and October 2008. Folic acid fortification of white wheat flour (150 μg/100 g flour) and grain products labeled enriched became mandatory in Canada in 1998 (30). Eight pregnant women (PW), between 30 and 36 wk of gestation, were consuming 1 mg/d of folic acid as part of a multivitamin and mineral supplement (MVM). The 24 remaining participants were all NPW (nonlactating) and were evenly divided into the following groups: not consuming a folic acid supplement (NPW-0); consuming 1 mg/d of folic acid as part of a MVM (NPW-1); and consuming 5 mg/d of folic acid as part of a MVM (NPW-5). All MVMs (Prenatal MVM, Life Brand; Materna, Nestle; Pregvit, Duchesnay; Jamieson Prenatal) included a source of vitamins B6 and B12. Women in the NPW-1 and NPW-5 groups were co-recruited with another study in which supplements were provided for 30 wk and verified by pill counts (31).

Study design and methods

Women participating in this cross-sectional study fasted overnight and refrained from taking their usual supplement (if using) on the morning of their study visit. During the visit, participants filled out a socio-demographic and supplement-use questionnaire and the validated Block Folic Acid/Dietary Folate Equivalents Screener (NutritionQuest) (32) Venous blood samples were collected into EDTA-lined tubes and processed within 2 h of collection. Aliquots of whole blood (100 μl) for determination of total RBC folate concentration by microbial assay were diluted 10 times with ascorbic acid (0.057 mol/L), mixed, incubated for 30 min at 37°C, and then frozen (33). Remaining whole blood was centrifuged (850 × *g*; 15 min at 4°C) to separate plasma and RBCs. The buffy coat was used for C677T methylenetetrahydrofolate reductase (*MTHFR*) genotyping (34). Sodium ascorbate solution (0.057 mol/L) was added to plasma samples prior to storage. Packed RBCs used for the later determination of folate forms by LC-MS/MS were washed twice and resuspended in an equal volume of NaCl (0.154 mol/L). The suspension was then diluted 20 times with ascorbic acid solution (0.028 mol/L, pH 4.2) and charcoal-treated human serum folate conjugase was added (100 μl human serum to ~100 nmol folate); this

hemolysate was incubated for 1 h at 37°C (35). Aliquots of whole blood and RBC hemolysates and plasma samples were stored at −80°C.

Total folate concentrations in whole blood hemolysates and plasma samples were measured by microbiologic assay, as described by Molloy and Scott (33), by using nonchloramphenicol-resistant *Lactobacillus rhamnosus* (ATCC7649; American Type Culture Collection) with folic acid to generate the standard curve. RBC folate content was calculated by using the analyzed whole blood folate concentration minus the plasma folate concentration corrected for hematocrit. A whole blood standard from the National Institutes for Biological Standards and Control (code 95/528, Hertfordshire, United Kingdom) with a certified folate content of 29.5 nmol/L was used in every assay. The overall inter-assay CV for the whole blood folate standard was 7.4% with a mean value of 28.2 nmol/L.

The concentrations of folate forms in RBCs [5-methyl-THF, pyrazino-s-triazine derivative of 4α-hydroxy-5-methyl-tetrahydrofolate (MeFox), tetrahydrofolate, 5-formyl-tetrahydrofolate, and 5,10-methenyl-tetrahydrofolate] were determined by using LC-MS/MS (27–29). RBC hemolysates (150 μL) were mixed with ammonium formate buffer and amended with a mixture of $^{13}C_5$-labeled folate internal standards. Sample clean-up was performed by using a 50-mg phenyl solid-phase extraction (SPE) 96-well plate (Bond Elut 96; Agilent Technologies) and an automated 96-probe SPE system (Caliper-Zephyr; Perkin Elmer) (29). Samples were eluted from the SPE plate with an organic elution buffer containing ascorbic acid and analyzed overnight by LC-MS/MS in positive-ion mode by using electrospray ionization on a Sciex API 5500 triple-quadrupole MS system (Applied Biosystems) coupled to a HP1200C LC system (Agilent Technologies). Chromatographic separation was achieved by using a Luna C-8 analytic column (Phenomenex) with an isocratic mobile phase and a total run time of 7 min (29). Three whole blood hemolysate bench quality control pools were analyzed in duplicate in every run, bracketing the study samples. The between-run imprecision (n = 5 d) for 3 quality control levels (2 levels for tetrahydrofolate, 5-formyl-tetrahydrofolate, and 5,10-methenyl-tetrahydrofolate) was 2.2–3.0% for 5-methyl-THF (20.6–37.1 nmol/L), 3.3–5.7% for MeFox (3.47–6.40 nmol/L), 4.6–8.2% for tetrahydrofolate (4.53–8.19 nmol/L), 5.5–5.6% for 5-formyl-tetrahydrofolate (2.97–5.60 nmol/L), and 3.3–6.9% for 5,10-methenyl-tetrahydrofolate (4.84–9.75 nmol/L). The limit of detection (nmol/L hemolysate) values were 0.06 (5-methyl-THF), 0.08 (MeFox), 0.2 (tetrahydrofolate), 0.2 (5-formyl-tetrahydrofolate), and 0.31 (5,10-methenyl-tetrahydrofolate).

Statistical analysis

Prior to statistical analysis, RBC folate data were log-transformed. A Pearson product-moment correlation coefficient was computed to assess the relation between RBC total folate concentrations determined by microbiologic assay and LC-MS/MS. Mean differences in RBC total folate concentrations, concentration of different folate forms, and the % distribution of different forms were determined by ANOVA. When a statistically significant difference was found, this was followed by pair-wise comparisons using the Tukey-Kramer method. Where indicated, individuals homozygous for the C677T *MTHFR* allele were removed from the analysis because it is known that these individuals accumulate non-methyl

folate forms (26,36,37). Statistical tests were performed by using SAS (version 9.1; SAS Institute), and $P < 0.05$ was considered statistically significant. Values in the text are expressed as means ± SDs.

Results

As illustrated in Figure 1, 32 women were enrolled in the study; however, 5 sample vials cracked during freezer storage (1 PW, 2 NPW-0, 2 NPW-1) and 26 samples were available for analysis. All women had a minimum of some college education, and there were no differences in age or education among the groups; however, there were differences in income and alcohol use (Table 1). There was no difference in dietary folate intake (naturally occurring and folic acid added as a fortificant) among groups, and all women reported consuming folic acid–fortified foods. Only 1 participant in the NPW-0 group consumed a vitamin and mineral supplement, and this consisted of calcium and vitamin D only. Except for 1 participant in the NPW-5 group who reported consuming her supplement every other day, all women consuming a supplement reported doing so daily. Mean duration of supplement use was longer in the PW group (46 ± 8 wk) compared with the NPW-1 and NPW-5 groups (30 ± 0 wk, $P < 0.001$).

RBC folate concentrations determined by microbiologic assay or LC-MS/MS (sum of folate forms) methods were strongly correlated ($n = 26$, $r = 0.91$, $P < 0.0001$). Mean RBC folate concentrations did not differ between the PW and NPW-1 groups as assessed by microbiologic assay or LC-MS/MS (Fig. 2). Women in the NPW-0 group had lower RBC folate concentrations than women in the NPW-1 and NPW-5 groups as assessed by LC-MS/MS and lower RBC folate concentrations than women in the NPW-5 group as assessed by microbiologic assay. There was no significant difference in mean RBC folate concentrations between the NPW-1 and NPW-5 groups, measured by either microbiologic assay or LC-MS/MS.

Distributions of folate forms in RBCs and the C677T *MTHFR* genotype for each participant are found in Supplemental Figure 1. Mean group values are presented in Table 2 with individuals homozygous for the C667T *MTHFR* allele excluded. No differences in 5-methyl-THF or MeFox were found between PW and supplemented NPW groups, expressed as a concentration or percentage distribution. Concentration of 5-methyl-THF in RBCs of women in the NPW-0 group was less than women in the NPW-5 group but did not differ from the NPW-1 group. No differences among the NPW-0, NPW-1, and NPW-5 groups existed in either 5-methyl-THF or MeFox as a percentage of total folate.

There were no statistically significant group differences in the sum of non-methyl folates or in the individual non-methyl folate forms expressed as a concentration or as a proportion of total folate among PW and NPW consuming 1 mg/d of folic acid. The concentration of the sum of all non-methyl folates (tetrahydrofolate, 5-formyl-tetrahydrofolate, and 5,10-methenyl-tetrahydrofolate) of the NPW-0 group was less than in the NPW-1 and NPW-5 groups as was the concentration of tetrahydrofolate. Nonetheless, no differences among the NPW-0, NPW-1, and NPW-5 groups existed in the sum of all non-methyl folate or in the individual non-methyl forms as a percentage of total RBC folate.

As expected, we found tetrahydrofolate and 5,10-methenyl-tetrahydrofolate in the RBCs of women who were C677T *MTHFR* homozygous regardless of supplementation status (Supplemental Fig. 1); however, we also found measurable concentrations of tetrahydrofolate (>0.2 nmol/L hemolysate) and 5,10-methenyl-tetrahydrofolate (>0.31 nmol/L hemolysate) in the RBCs of women who had both a wild-type and heterozygous genotype. Three of 5 NPW taking 0 mg of folic acid had measurable concentrations of tetrahydrofolate and 1 had 5,10-methenyl-tetrahydrofolate. Even after excluding C677T *MTHFR* homozygotes, most of the PW taking 1 mg of folic acid (4/6) had measurable concentrations of 5-formyl-tetrahydrofolate in RBCs, whereas there was only 1 of 4 women in the NPW-1 group who had measurable concentrations.

Discussion

There is strong evidence in the literature that folate requirements increase during pregnancy to support rapid fetal and uteroplacental growth (38,39), yet the changes that occur to support this rapid rate of anabolic activity have not been fully explored. In the present study, we did not find any difference in the distribution of folate forms in RBCs between the PW and NPW-1 groups, suggesting that there is little evidence folate is preferentially used for purine and pyrimidine synthesis for erythropoiesis in pregnancy. If a physiologic response in folate metabolism occurred to accommodate increased DNA/RNA synthesis, we would anticipate a shift in folate metabolism from re-methylation of homocysteine to methionine to purine and pyrimidine biosynthesis resulting in a corresponding increase in non-methyl folates forms.

To our knowledge, this is the first time the RBC folate forms between PW and NPW, or NPW consuming different concentrations of supplemental folic acid, have been directly compared. As far as we are aware, only 2 groups have examined the distribution of folate forms in blood during pregnancy. Obeid et al. (40) reported that the serum total folate, 5-methyl-THF, 5-formyl-tetrahydrofolate, and tetrahydrofolate concentrations of PW supplemented with folic acid (400 μg/d) immediately prior to delivery were higher than concentrations in PW not consuming a folic acid supplement. However, as with the results of the current study, the distribution of the different folate forms as a relative percentage of total folate remained unchanged between the folic acid–supplemented PW and the nonsupplemented PW. In PW supplemented with folic acid, 92.9%, 0.94%, and 8.3% of serum total folate was in the 5-methyl-tetrahydrofolate, formyl-tetrahydrofolate, and tetrahydrofolate forms, respectively, compared with 85.4%, 0.95%, and 11.7% among nonsupplemented PW.

Houghton et al. (35) reported that the RBC folate forms of PW (an equal number of women with CC, CT, and TT C677T *MTHFR* genotypes) consuming 1 mg/d folic acid contained a significant proportion of RBC folates in the tetrahydrofolate form (55–59.5%). These data differ from those in the present study where only a small percentage of total folates were found as tetrahydrofolate regardless of whether women were pregnant or were supplemented with folic acid. In the former study, it was postulated that the elevated tetrahydrofolate concentrations reflected cellular uptake of high concentrations of circulating unmetabolized folic acid early in the erythroid lineage or alternatively reflected the elevated concentration

of high-affinity binding proteins during pregnancy, which are believed to protect labile tetrahydrofolate from degradation (41,42). Results from the current study suggest, however, that the elevated tetrahydrofolate concentrations in the latter study likely reflected an analytic aberration, potentially resulting from folate form interconversions during analysis, i.e., between 5,10-methylene-tetrahydrofolate and tetrahydrofolate, causing an overestimation of tetrahydrofolate (35,43).

There is considerable evidence in the literature suggesting that physiologic shifts in folate metabolism occur in response to certain conditions such as dietary deficiencies, environmental exposures, and abnormal anabolic conditions such as cancer. For example, dietary deficiencies of methionine (in kwashiorkor), folate, or vitamin B-12 result in a reduction of *S*-adenosylmethionine and a corresponding rise in 5-methyl-THF because of the reduced activity of methionine synthase, which converts 5-methyl-THF to tetrahydrofolate (12). *S*-adenosylmethionine concentrations play an important regulatory role in folate metabolism, and low concentrations stimulate MTHFR activity to shunt folate to 5-methyl-THF and divert folate away from purine and pyrimidine biosynthesis (12). Correction of methionine, folate, or vitamin B-12 deficiency will reduce 5-methyl-THF, increase *S*-adenosylmethionine, and hence increase formyl-tetrahydrofolate concentrations (substrate for purine biosynthesis) and conversion of 5,10-methenyl-tetrahydrofolate to thymidylate (for DNA biosynthesis) (13). It was reported that higher proportions of formyl-tetrahydrofolate and tetrahydrofolate and lower proportions of 5-methyl-THF are found in colorectal tumors compared with normal mucosa with no correlation to RBC folate concentration (15). This shift in folate forms was accompanied by a decrease in global DNA methylation, thought to be an important mechanism in the pathogenesis of colorectal cancer (16). In the current study, we did not find evidence that RBC folate, reflecting metabolism earlier in erythropoiesis, was shunted toward purine and pyrimidine biosynthesis during pregnancy; however, we cannot discount the possibility that this may occur in other tissues.

Limitations to this study include the small sample size, although results obtained by using our new, sophisticated LC-MS/MS technique indicate that differences between the relative distribution of RBC folate forms, our main outcome, were small. To determine the small effect size observed between PW and the NPW-1 group, e.g., for % 5-methyl-THF, % sum of non-methyl-THFs, and % tetrahydrofolate, would require sample sizes of 72, 512, and 364, respectively. Whether the small differences noted are clinically relevant and warrant larger studies is uncertain. Recent evidence suggests that it may take longer than 1 complete turnover of all RBCs (120 d or 17 wk) to reach a new steady-state RBC folate concentration after commencement of folic acid supplementation (44,45). PW in our study consumed folic acid supplements longer than NPW (46 ± 8 wk vs. 30 ± 0 wk, $P < 0.001$), which leaves open the possibility that differences in the main outcome (relative distribution of RBC forms) could have been due to differences in the duration of folic acid supplementation. Because no differences were found, we do not believe this limitation biased our study conclusions. Finally, it should be noted that all women in this study, including those in the NPW-0 group, were consuming folic acid–fortified foods. It is unclear whether the results presented herein can be generalized to reproductive-age women in countries where folic acid fortification is not present.

In conclusion, the results of this study suggest that there is little evidence that folate is being preferentially used early in erythropoiesis for purine and pyrimidine synthesis during pregnancy because we found no increase in the proportions of non-methyl folate forms. Furthermore, there is little evidence that folic acid supplementation at high concentrations (1 and 5 mg/d) alters the distributions of the folate forms in RBCs. Given our small sample size, it is unclear whether our results can be generalized to all reproductive-age women, particularly those not consuming folic acid–fortified foods, with baseline RBC folate concentrations lower than reported herein. The data presented herein do, however, provide the basis for further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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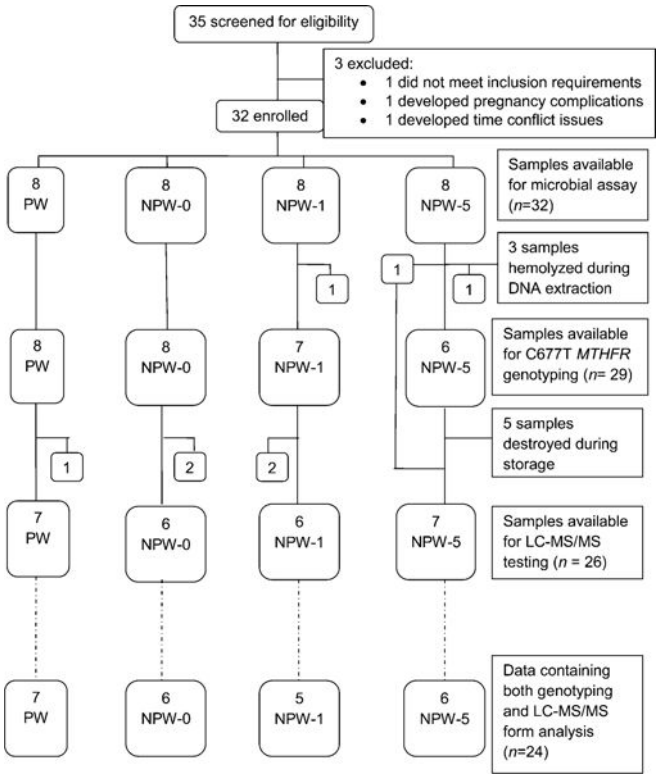

FIGURE 1.

Disposition of study participants and availability of samples for analysis. *MTHFR*, methylenetetrahydrofolate reductase; NPW-0, non-pregnant women not consuming a folic acid supplement; NPW-1, non-pregnant women consuming a 1 mg/d folic acid supplement; NPW-5, non-pregnant women consuming a 5 mg/d folic acid supplement; PW, pregnant women (consuming a 1 mg/d folic acid supplement).

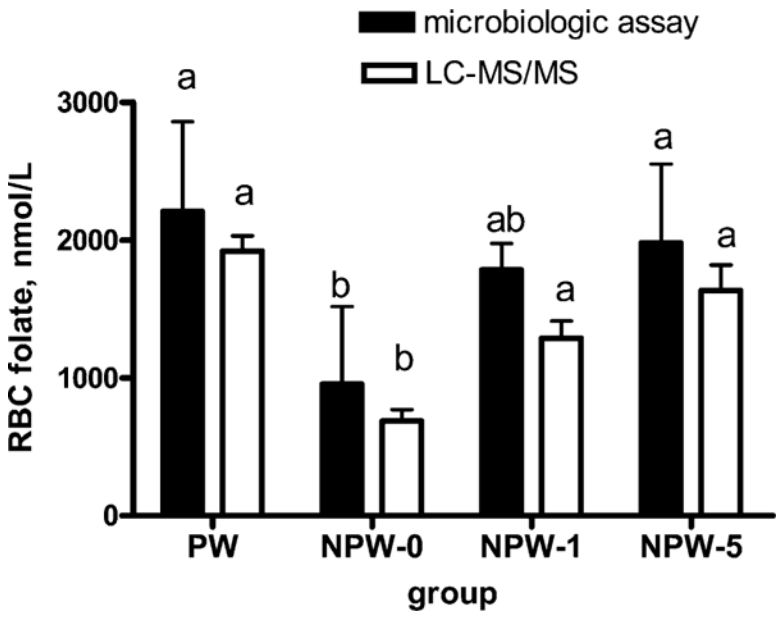

FIGURE 2.

RBC total folate concentrations in PW consuming 1 mg/d of folic acid and nonpregnant women consuming 0, 1, or 5 mg/d of folic acid as determined by microbiologic assay or LC-MS/MS. Women homozygous for the C677T *MTHFR* genotype were not included in these analyses. Values are means ± SDs; PW ($n = 6$), NPW-0 ($n = 5$), NPW-1 ($n = 5$), NPW-5 ($n = 7$). Within an assay, means without a common letter differ, $P < 0.05$. *MTHFR*, methylenetetrahydrofolate reductase; NPW-0, nonpregnant women not consuming a folic acid supplement; NPW-1, nonpregnant women consuming a 1-mg/d folic acid supplement; NPW-5, nonpregnant women consuming a 5-mg/d folic acid supplement; PW, pregnant women.

TABLE 1

Characteristics of pregnant and nonpregnant female participants consuming different amounts of supplemental folic acid[1]

	PW (*n* = 7)	NPW-0 (*n* = 6)	NPW-1 (*n* = 6)	NPW-5 (*n* = 7)	*P*
Age, *y*	32 ± 2.2	28 ± 4.7	31 ± 4	36 ± 6.6	0.10
Gestational age, *wk*	33.4 ± 2	n/a	n/a	n/a	
Race, *n (%)*					0.33
White	6 (85)	2 (33)	5 (83)	2 (28)	
Nonwhite	1 (15)	4 (67)	1 (17)	5 (72)	
Income,[2] *n (%)*					0.0207
<60,000	4 (57)	6 (100)	5 (83)	2 (29)	
≥60,000	3 (43)	0 (0)	1 (17)	5 (71)	
Education, *n (%)*					0.45
College or university	4 (57)	1 (17)	2 (33)	3 (43)	
<Baccalaureate	3 (43)	5 (83)	4 (67)	4 (57)	
Alcohol use, *n (%)*					0.0396
None	7 (100)	2 (33)	1 (17)	2 (29)	
≤1/wk	0 (0)	1 (17)	2 (33)	4 (57)	
≥2/wk	0 (0)	3 (50)	3 (50)	1 (14)	
C677T *MTHFR* genotype, *n (%)*					0.47
CC	1 (14)	3 (50)	1 (20)	4 (67)	
CT	5 (72)	2 (33)	3 (60)	2 (33)	
TT	1 (14)	1 (17)	1 (12.5)	0 (0)	
Missing			1	1	
Dietary folate intake, m*g DFE/d*	480 ± 230	590 ± 140	390 ± 80	480 ± 190	0.33
Plasma folate,[3] *nmol/L*	80 ± 13[ab]	46 ± 33[b]	54 ± 9[ab]	92 ± 34[a]	0.0095
RBC folate,[3] *nmol/L*	2130 ± 630[a]	890 ± 530[b]	1660 ± 350[a]	1980 ± 570[a]	0.0022

[1] Values are means ± SDs unless otherwise indicated, $n = 26$. Labeled means in a row without a common letter differ, $P < 0.05$. DFE, dietary folate equivalent; *MTHFR*, methylenetetrahydrofolate reductase; n/a, not applicable; NPW-0, nonpregnant women not consuming a folic acid supplement; NPW-1, nonpregnant women consuming a 1-mg/d folic acid supplement; NPW-5, nonpregnant women consuming a 5-mg/d folic acid supplement; PW, pregnant women (consuming a 1-mg/d folic acid supplement).

[2] Income is reported in Canadian dollars.

[3] Plasma and RBC folate concentrations were measured by microbiologic assay.

TABLE 2

Forms of folate and their relative distributions in RBCs of pregnant and nonpregnant female participants consuming different amounts of supplemental folic acid[1]

	PW (*n* = 6)	NPW-0 (*n* = 5)	NPW-1 (*n* = 4)	NPW-5 (*n* = 6)	*P*
Genotype					
C677T *MTHFR* CC, *n*	1	3	1	4	
C677T *MTHFR* CT, *n*	5	2	3	2	
Folate form					
5-methyl-THF, *nmol/L*	1600 ± 230[a]	570 ± 160[b]	1110 ± 225[ab]	1380 ± 420[a]	0.0002
% Total folate	83.5 ± 2.2	83.2 ± 0.9	82.4 ± 0.8	84.3 ± 1.1	0.25
MeFox, *nmol/L*	261 ± 53[a]	102 ± 25[b]	198 ± 27[a]	240 ± 64[a]	0.0002
% Total folate	13.5 ± 1.1	15 ± 0.6	15 ± 1.6	14.4 ± 1.5	0.21
Sum of non-methyl folate,[2] *nmol/L*	48 ± 50[a]	4 ± 6[b]	36 ± 33[a]	19 ± 11[a]	0.0303
% Total folate	3 ± 2.7	0.6 ± 0.7	2.4 ± 2	1.1 ± 0.6	0.24
Tetrahydrofolate, *nmol/L*	26 ± 19[a]	4 ± 4[b]	24 ± 20[a]	13 ± 7[a]	0.0408
% Total folate	1.3 ± 0.9	0.5 ± 0.5	1.6 ± 1.2	0.7 ± 0.4	0.47
5-formyl-tetrahydrofolate,[3] *nmol/L*	7 ± 4	0 ± 0	5 ± 0	0 ± 0	n/a
% Total folate	0.3 ± 0.25	0 ± 0	0.3 ± 0	0 ± 0	n/a
5,10-methenyl-tetrahydrofolate, *nmol/L*	26 ± 38	1 ± 3	11 ± 11	5 ± 5	0.67
% Total folate	1.4 ± 2.1	0.2 ± 0.3	0.7 ± 0.7	0.3 ± 0.2	0.61

[1] Values are means ± SDs unless otherwise indicated. Labeled means in a row without a common letter differ, $P < 0.05$. MeFox, 4α-hydroxy-5-methyl-tetrahydrofolate; *MTHFR*, methylenetetrahydrofolate reductase; NPW-0, nonpregnant women not consuming a folic acid supplement; NPW-1, nonpregnant women consuming a 1-mg/d folic acid supplement; NPW-5, nonpregnant women consuming a 5-mg/d folic acid supplement; PW, pregnant women (consuming a 1-mg/d folic acid supplement); 5-methyl-THF, 5-methyltetrahydrofolate.

[2] Sum of tetrahydrofolate + 5-formyl-tetrahydrofolate + 5,10-methenyl-tetrahydrofolate.

[3] Insufficient cell size for analysis of 5-formyl-tetrahydrofolate.